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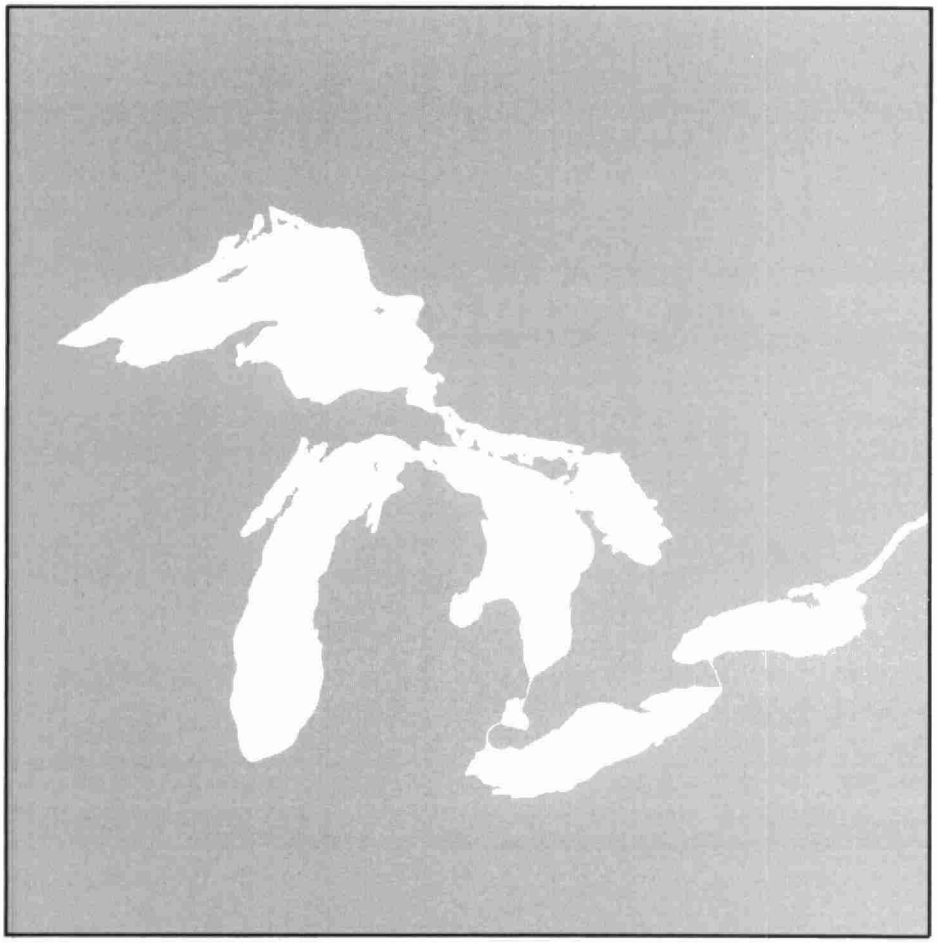

Contaminated Sediments in Great Lakes

Areas of Concern

Volume 2: Laboratory Sediment Bioassays

August 1988

Canada Ontario

Canada-Ontario Agreement Respecting Great Lakes Water Quality
L'Accord Canada-Ontario relatif à la qualité de l'eau dans les Grand Lacs

CONTAMINATED SEDIMENTS IN GREAT LAKES AREAS OF CONCERN

VOLUME 2: LABORATORY SEDIMENT BIOASSAYS

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FOREWORD

This document has been prepared for the COA Polluted Sediment Committee to assist Remedial Action Plan (RAP) Teams during the preparation of their RAP documents. The COA Polluted Sediment Committee is comprised of federal and provincial representatives who are seeking to achieve an enhanced understanding of the environmental impacts of polluted sediments, as well as appropriate remedial actions, by coordinating research and investigation efforts.

The Committee will be providing continued assistance to RAP Teams throughout the RAP process preparing general advisory documents, and by consulting directly with individual RAP Teams.

Volume 1, entitled "Initial Assessment" (prepared by D. Boyd, T. Lomas and L. Sarazin) contained recommendations concerning the minimum data requirements for assessment of contaminated sediments at Areas of Concern (i.e., sediment chemistry, sediment sources, benthic enumeration and tissue contaminant concentrations in benthos). It also identified the probable need for further assessment of specific ecosystem components in order to identify the most appropriate and effective course of remedial action. These more detailed assessments were identified as being likely to fall under the headings of laboratory bioassessment, sediment transport and food web assessment.

This second document contains further detail concerning laboratory bioassessment of contaminated sediments. It provides a definition of bioassays and the rationale for various experiments and their corresponding endpoints. It also summarizes the current Ontario Ministry of the Environment protocol and future directions in sediment bioassay enhancement and development.

The aim of this second document is to provide RAP Teams with a uniform, general information base regarding sediment bioassays, and to describe those procedures which the COA Polluted Sediment Committee considers to be sufficient for biological assessment of contaminated sediments.

AVANT-PROPOS

Le présent document a pour but d'aider les équipes de mise en oeuvre des plans d'assainissement quand elles ont à rédiger des rapports et autres sur ceux-ci. Il est l'oeuvre du comité sur les sédiments pollués créé dans le cadre de l'Accord Canada-Ontario relatif à la qualité de l'eau dans les Grands Lacs. Ce comité, qui réunit les représentants des gouvernements fédéral et provincial, s'efforce, en coordonnant les activités de recherche et d'investigation, de parvenir à une meilleure compréhension des effets des sédiments pollués sur l'environnement et d'établir les mesures correctrices appropriées.

Le comité facilitera la tâche aux équipes tout au long de la mise en oeuvre des plans en préparant divers documents d'ordre général pour les guider et en les consultant individuellement.

Le volume 1, intitulé Initial Assessment (D. Boyd, T. Lomas et L. Sarazin), renfermait des recommandations sur les données minimales à recueillir au moment de l'évaluation des sédiments pollués dans les divers secteurs de préoccupation (la chimie des sédiments, leur provenance, le benthos et les concentrations de polluants chez celui-ci). Il y était également question de la quasi-nécessité d'évaluer plus avant certains écosystèmes avant de pouvoir établir les mesures correctrices les plus appropriées et les plus efficaces. Ces évaluations plus poussées, disait-on, prendraient le plus souvent la forme d'évaluations biologiques en laboratoire et d'évaluations du réseau alimentaire et du transport des sédiments.

Ce deuxième document contient des précisions sur l'évaluation biologique, en laboratoire, des sédiments pollués. On y définit les essais biologiques et y explique la raison d'être de diverses expériences ainsi que leurs objectifs. On y résume aussi le protocole actuel du ministère de l'Environnement de l'Ontario en matière d'essais biologiques sur les sédiments et y donne l'orientation future à cet égard.

Le document a un double but : fournir aux équipes chargées des divers plans d'assainissement une base de données uniforme sur ces essais, et décrire les modalités que le comité juge suffisantes pour l'évaluation biologique des sédiments pollués.

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1.0 INTRODUCTION

Sediment bioassays are studies aimed at identifying sediment-related environmental impacts to aquatic biota. The studies provide information on whether bioavailable contaminants present in the sediment are toxic and/or bioaccumulative. While chemical measurements of contaminated sediments can identify the presence of toxic pollutants, they generally do not provide an adequate indication of the availability or effect of those contaminants to the biota. By testing organisms that inhabit different ecological niches, such as sediment, sediment-water interface and water column, information on the route of contaminant exposure can also be obtained.

Sediment bioassays then, are an important part of sediment assessment because they provide information on sensitivity of aquatic biota and their responses to the bioavailable portion of contaminants in sediments.

The following section provides the rationale for sediment bioassays and briefly describes the study endpoints that may be achieved.

1.1 Rationale

Sediment bioassays provide the opportunity to study aquatic biota and their responses to contaminants in sediments while in a relatively controlled environment. Laboratory based bioassays can achieve a level of control unobtainable in the field.

Physical and chemical factors such as temperature, light, dissolved oxygen and pH can alter the response of the test organism and can be readily monitored and maintained at prescribed values in the laboratory.

Biological factors such as organism age, size, general health, reproductive state and previous history of exposure to contaminants can significantly effect the sensitivity of the test organism. These variables can be controlled and their effects minimized in the laboratory.

Chemical measurements alone are generally insufficient for predicting, with assurance, the toxicity of mixtures of pollutants to the biota. Biological responses provide integrated products of the complex interactions among pollutants present, including those not measurable or otherwise known to be present.

Sediment bioassays assist in the interpretation of chemical measurements of sediment-associated contaminants. They can also generate information on the acute and chronic impacts of sediments that may be influencing benthic communities in the field. In this respect, it is important to relate bioassay results with the natural field conditions (e.g. compare bioassay results with results of the initial assessment, described in Volume 1). The initial assessment includes sediment quality, benthic community structure and contaminant analyses of benthic invertebrates. Sediment bioassays may identify a sediment problem but these results must be compared to and validated by field results.

1.2 Sediment Bioassay Endpoints

Several endpoints may be measured during a sediment bioassay. Acute and chronic responses of the test organism, as well as bioaccumulation (retention of contaminants) may provide an indication of the sediment quality.

Acute Lethality:

The endpoint of an acute lethality test is mortality within a specified, short-term exposure period. An acute lethality test provides a rapid and relatively inexpensive screening tool that identifies whether pollutants are immediately toxic to aquatic biota. An example of this type of sediment bioassay is the 10 day toxicity test with the infaunal Phoxocephalid amphipod (Rhepoxynius abronius) currently being proposed for marine systems (by USEPA). Acutely lethal responses provide strong evidence that sediment contamination will adversely affect the aquatic biota native to the study site and may result in the impoverishment of benthic communities.

Chronic Toxicity:

A chronic toxicity test indicates sublethal effects of contaminants in the sediment on the test organism within a specified exposure period that exceeds the short-term (acute) interval, and does not necessarily result in mortality. Endpoints that have been considered include growth inhibition, respiratory changes and reproductive impairment. The most useful tests examine life cycle effects and determine the response of the most sensitive stages of the organism. Where benthic community structure in the field and/or chemical analyses suggest that sediment contamination may be a problem, but sediments do not elicit acute mortality, chronic tests can demonstrate effects on benthic organisms at a more subtle level.

Bioaccumulation:

Bioaccumulation is a measure of the uptake and retention of pollutants from the contaminant source by the test organism. The source may be sediment and/or water and refers to uptake by both ingestion and absorption. Bioaccumulation can be expressed as an absolute change in tissue concentrations for a specified exposure period (the rate of bioaccumulation) or the ratio of specified contaminant concentrations in biota to those in the organism's environment (e.g. water, sediment, porewater). If bioaccumulation occurs, there is concern that contaminants in sediments may be transferred into the food web.

2.0 SEDIMENT BIOASSAYS

During the fall of 1985, the Ontario Ministry of the Environment (OMOE) developed a sediment bioassay procedure to assess both acute toxicity and bioaccumulation of contaminants in sediments. Other techniques have also been suggested and listed by the International Joint Commission. These tests include: Microtox R, Algal Fractionation Bioassay, Daphnia magna (chronic and acute), Fathead Minnow Bioaccumulation, Fish Embryo Larval Test, Benthic Bioassay (Chironomus tentans, Hexagenia limbata, Hexagenia azteca) and Ames Test. A more detailed description of these

tests is available in the International Joint Commission, Sediment Subcommittee's 1987 Draft Report entitled "Guidance on Assessment and and Remediation of Contaminated Sediment Problems in the Great Lakes".

The following information presents an outline of the current OMOE Sediment Bioassay Protocol.

2.1 Ontario Ministry of the Environment (OMOE) Sediment Bioassay Protocol

The OMOE experiments are run as static beaker tests, using two types of aquatic biota: 3-4 month old fathead minnows, Pimphales promelas (to assess effects of contaminated sediment on a water column organism) and 2nd year nymphs of the burrowing mayfly, Hexagenia limbata (to assess effects of contaminated sediment on a sediment-dwelling organism). The organisms are placed in jars (2 litre) with dechlorinated water and sediment (4:1 ratio) and during the 10 day exposure, biological responses are monitored. At the end of the experiment, mortality is tabulated and all living biota are harvested for contaminant analyses.

OMOE has carried out bioassays on sediments from several RAP and MISA sites. The protocol is currently under review and future research endeavours are being suggested.

Table 1 provides the present sediment bioassay methods, reasons why these methods were selected and current/future research being considered in order to address specific concerns.

2.1.1 Selection of Controls

Controls are very important and necessary for proper interpretation of bioassay results. Two types of control sediments are selected for the OMOE Sediment Bioassay Protocol and these are:

- Sediment where test organisms were collected from or cultured in.
- Control site from study location, upstream or removed from the pollution sources being assessed.

2.2.2 Data Interpretation

Data interpretation involves comparing bioassay results from test sediments to results from:

- replicate test sediments to address variability among replicates
- control sediments that organisms were collected from or cultured in
- upstream control sediments or sediments removed from pollution source being assessed.

Statistically significant ($P<0.10$- $P<0.05$) differences between test and control sediments for the various endpoints indicate that test sediments have negatively impacted the biota. Control mortality is monitored and must not exceed 10% for the validation of test results.

3.0 SUMMARY OF FUTURE DEVELOPMENT AND REVISIONS OF SEDIMENT BIOASSAY

To enhance the utility of sediment bioassays for assessing field toxicity, both chronic and acute, and to estimate contaminant bioavailability by monitoring bioaccumulation, the Ministry is engaged in several aspects of research.

As outlined in Table 1, studies are currently underway to identify the significance of sediment manipulation on the bioassay results.

These include:

- sieving
- homogenization
- bioassay assembly
- period of equilibration
- sediment: water ratio
- sediment storage
- exposure time

Biological constraints on the sensitivity of the bioassay will necessitate investigations on the importance of:

- organism density
- feeding
- habitat preference
- predator-prey interactions

An examination of chronic toxicity tests to measure sublethal responses is of high priority. Chronic tests, conducted for the full or partial duration of the organism's life cycle, will include measurements of:

- growth inhibition
- reproductive impairment

Further information on the detail and estimated time frame for these research directions will be provided in an interim report by Krantzberg (1988).

4.0 SUMMARY

Sediment bioassays should be considered as a routine method for assessing sediment quality. Sediment bioassay results may identify a sediment problem but they are only one component of a comprehensive evaluation of sediment contamination and results must be compared with physical and chemical information obtained on sediment quality, benthic community structure and bioaccumulation.

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Table 1: Present OMOE Protocol and Future Research

Method	Explanation	Research and Development
1. Sediment collection - grab sampler technique	Ponar grab sampler was used for the In-Place Pollutants Program field study. Maintains consistency with collection. Allows for adequate sample volume for a wide range of substrates.	
2. Store sediment in polyethylene bag at 4°C for no more than 2 weeks	4°C has been shown to be the best temperature for sediment storage - least disruption of sediment chemistry.	The effect of the duration of sample storage on the response of the bioassay organism will be examined in 1988.
3. Sieve sediment through sediment wash bucket (U.S. #30) (in air)	To remove large particles and debris. To homogenize sediment for consistency.	Sediment homogenity insures that all organisms receive the same dose of contaminants. Homogenization, however, can alter bioavailability. Comparisons between the toxicity of well mixed sediments and intact cores is under consideration and will be examined during 1988.
4. Jars for experiment: 2 l clear glass jars	Mason jars are easy to obtain and inexpensive. The 1 l volume allows for approximately 3 cm depth of sediment (200 ml). This depth will allow the mayfly nymphs to burrow.	
5. Fill jars with 1300 ml dechlorinated water, 325 ml sieved sediment (4:1 v:v)	4:1 ratio has been most frequently used in experiments of this nature.	The sensitivity of different test organisms to alterations in this ratio will be further examined.

Table 1: Present OMOE Protocol and Future Research (Cont'd)

Method	Explanation	Research and Development
6. Allow sediment to settle over 24 hours	Adding the water may disturb sediments and necessitate a period to reduce turbidity (24 hrs).	The settling time required to achieve steady-state with respect to contaminant distribution and the effect of settling time on the bioassay results will be investigated. Preliminary findings will be available in 1988.
7. Aerate for one hour before introducing organisms	Increases dissolved oxygen in water.	
8. Continue to aerate during experiment	To prevent anoxia.	
9. Organism selection - water column - sediment	Water column organisms will reflect toxicity associated with contaminated suspended sediment and dissolved contaminants. Sediment organisms burrow into sediment, ingesting quantities of contaminated material.	Further consideration of several water column and benthic organisms will focus on the different routes of exposure that are most likely to be of importance to different species (e.g. filter feeders, deposit feeders, predators).
10. Assemble a minimum of 3 replicate jars of fathead minnows (3-4 month old) and 3 replicate jars of nymphs of the burrowing mayfly (2nd year)	3 replicates are the minimum number to be used to achieve statistical significance.	
11. Use 10 organisms per jar	10 organisms allow for an assessment of the variability of the results. Also enables bioaccumulation studies to be carried out (where analyses requires a minimum biomass).	The feasibility of increasing the number of organisms per container will be examined by monitoring the effects of density on the response of the bioassay. Depending upon the final choice of maximum acceptable density, the number of replicate containers may be increased.

Table 1: Present OMOE Protocol and Future Research (Cont'd)

Method	Explanation	Research and Development
12. Run experiment at 20°C (in a temperature controlled water bath)	Selected organisms can easily live at 20°C. Metabolic activity greater than at lower temperature, therefore response to pollutants likely to be more rapid.	
13. Place lid on jars (not air tight)	Reduce evaporation and minimize the introduction of air-borne contaminants, if present.	
14. Use natural lighting	Simple, easy to maintain.	For the development of partial or full-life-cycle tests (see Section 3) control of photoperiod may be necessary.
15. Measure water pH and dissolved oxygen	To observe whether any change occurs during experiment and assist in data interpretation.	If chronic tests are used, pH may have to be maintained by titration with acid or bases.
16. Monitor experiment each day. Observations include: - mortality - behaviour (stress) (remove dead organisms)	Behavioural observations and daily monitoring assist with sediment bioassay interpretation.	The use of additional endpoints to monitor are being considered and are outlined in Section 3.
17. Top up water during experiment	To maintain 4:1 water: sediment ratio and minimize artifacts due to increasing concentrations in the water column.	

Table 1: Present OMOE Protocol and Future Research (Cont'd)

Method	Explanation	Research and Development
18. Day 10 - harvest organisms and store remaining biota (frozen) for contaminant analyses.	10 days has been shown to be acceptable for monitoring changes in tissue concentration (bioaccumulation, toxicity) Bioaccumulation studies may be carried out on frozen biota.	Different bioassay durations are being examined. A holding period in clean water in order to allow organisms to evacuate gut contents may be introduced for improved accuracy in the measurements of tissue concentrations. Recommendations will be made in 1988.


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